ERJ Express. Published on June 23, 2011 as doi: 10.1183/09031936.00023211

Comparative cost and performance of LED-microscopy in HIV-TB co-infected patients

Andrew WHITELAW[1*], Jonathan PETER[2*], Hojoon SOHN[4], Debbi VILJOEN[1], Grant THERON[2], Motasim BADRI[3], Virginia DAVIDS[2], Madhukar PAI[4], Keertan DHEDA[2,5,6]

[1] Division of Medical Microbiology, University of Cape Town, Cape Town, South Africa
[2] Lung Infection and Immunity Unit, Division of Pulmonology and Clinical Immunology and UCT Lung Institute, Department of Medicine, University of Cape Town, Cape Town, South Africa,
[3] Clinical Research Support Unit, Department of Medicine, University of Cape Town, Cape Town, South Africa,
[4]Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Canada,
[5] Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, Cape Town, South Africa
[6] Centre for Infectious Diseases and International Health, Department of Infection, University College London Medical School, London, United Kingdom
* Equally contributing 1st authors

Running Title: LED microscopy in HIV-TB co-infection

Corresponding author:
K Dheda, Department of Medicine, H floor (Old Main Building), Groote Schuur Hospital, Observatory, Cape Town, South Africa, 7925. Tel: +27 21 4066509, Fax: +27 21 4486815, mobile: (0)845577754 email: keertan.dheda@uct.ac.za

Alternative corresponding author:

A Whitelaw, Department of Medical Microbiology, University of Cape Town.

Current address: Department of Medical Microbiology, NHLS C18 laboratory, Groote Schuur Hospital. Observatory, Cape Town, South Africa, 7925. Tel: +27 21 4045137, Fax: +27 21 4486815, mobile: (0)823756297; email: andrew.whitelaw@uct.ac.za

Abstract

Objectives: Light-emitting-diode microscopy(LED) has recently been endorsed by the WHO. However, it is unclear if LED is as accurate and cost-effective as Ziehl-Neelsen(ZN) or mercury-vapour-fluorescence-microscopy(MVFM) in TB-HIV co-infected persons.

Methods: Direct and concentrated sputum smears from TB suspects were evaluated using combinations of LED, ZN-microscopy and MVFM. Median reading time per slide was recorded and a cost analysis performed. Mycobacterial culture served as the reference standard.

Results: 647 sputum samples were obtained from 354 patients[88(29.8%) HIV-infected and 161(26%) culture-positive for *M.tb*]. Although overall sensitivity of LED compared to ZN-microscopy or MVFM was similar, sensitivity of all three modalities was lower in HIV-infected patients. In the HIV-infected group the sensitivity of LED was higher than ZN using unconcentrated samples(46 vs. 39%;p=0.25), and better than MVFM using concentrated samples(56 vs. 44;p=0.5). A similar trend was seen in the CD4 count <200cells/mm^3 subgroup. Median(IQR) reading time was quicker with LED compared to ZN-microscopy[1.8(1.7-1.9) vs. 2.5(2.2.-2.7) minutes,p=<0.001]. Average cost per slide read was cheaper for LED-microscopy(U$1.63) compared to ZN-microscopy(U$2.10).

Conclusions: Among HIV-TB co-infected patients LED-microscopy was cheaper and performed as well as ZN-microscopy or MVFM independent of the staining(ZN or Auramine-O) or processing method used.

Keywords: smear microscopy, LED, TB, HIV

Abstract word count: 191

Background

Despite numerous advances, microscopy remains the cornerstone of tuberculosis (TB) diagnosis particularly in developing countries[1]. Fluorescent stains increase sensitivity by up to 10% over carbol-fuchsin-based stains and reduce the time required to read smears[2]. However, fluorescent microscopes using mercury vapour lamps (MVL) are relatively expensive, have a short life span (~250 hours), require a reliable electricity supply, and replacement bulbs may be difficult to obtain [3]. These factors have delayed the wider implementation of fluorescent microscopy, and have led to an interest in fluorescent microscopy using light emitting diodes (LEDs). LEDs have a lifespan of up to 50 000 hours, may be battery operated and do not require a dedicated darkroom [3]. These advantages together with a potential cost benefit make LED technology particularly appealing for high burden resource-limited settings [4]

In 2010, the WHO issued as policy statement, recommending that conventional fluorescence microscopy be replaced by LED microscopy using auramine staining in all settings where fluorescence microscopy is currently used, and that LED microscopy be phased in as an alternative for conventional ZN light microscopy in both high and low-volume laboratories [5]. A meta-analysis commissioned by WHO, of published and unpublished data, found that LED microscopy was significantly more sensitive (~6%) without appreciable loss in specificity when compared to direct ZN microscopy [5]. Other studies have also shown good concordance between the performance of LED and conventional fluorescent microscopy [6-8]. However, these studies had a low proportion of HIV-infected participants.

Given the lower concentration of bacilli in the sputa of TB-HIV co-infected patients and the relevance to large parts of Africa where ZN microscopy is the norm, it remains unclear whether LED microscopy performs as well as other microscopy methods in samples obtained from HIV-infected patients. The aim of this study was to assess the performance and cost of LED fluorescence microscopy compared to conventional light microscopy and MVL fluorescent microscopy in TB-HIV co-infected patients.

Methods

Patients

Consecutive ambulant patients with suspected TB (≥ 18 years of age) were recruited from 2 primary care clinics in Cape Town, South Africa, during 2009. Informed consent was obtained from all participants and the study was approved by the University of Cape Town Human Research Ethics Committee. HIV and CD4 count testing (if HIV-infected) was performed in all consenting study participants. Two expectorated sputum samples were collected from each patient where possible.

Laboratory processing

Two direct smears were prepared from each sample prior to N-acetyl-L-cysteine / NaOH decontamination [9]. One of these was Ziehl-Neelsen stained , whereas the other was stained with auramine-O and read at 200X magnification using the Lumin^{TM} (LW Scientific, Lawrenceville, GA, USA) LED attachment fitted to a light microscope. Thereafter, the specimens were decontaminated and centrifuged and 0.5ml of the deposit inoculated into a Mycobacterial Growth Indicator Tube (MGIT; Becton Dickinson Diagnostics, USA). Two further smears were prepared from the deposit, both Auramine-O stained, one read with the LED attachment and the other with a conventional MVL microscope (Zeiss Axioskop). Batches (maximum 20 slides) with varying proportions of smear +/- slides were read by a qualified medical technologist blinded to other microscopy and culture results. Total time taken to read each batch was recorded and the average time to read each slide calculated. Positive slides were graded according to WHO guidelines and the grading of the auramine stained smears converted to account for the difference in magnification between fluorescent and light microscopy [10].

Cultures positive for acid fast bacilli were identified as *M. tuberculosis* complex using either an in-house PCR method [11], or the Hain MTBDRplus® assay (Hain LifeSciences, Nuhren, Germany) if susceptibility testing had been requested.

Analysis

The reference standard was at least one positive MGIT culture for *M. tuberculosis*. Test accuracy results were computed as sensitivity and specificity, along with 95% confidence intervals (CI). Categorical variables were compared using the χ^2 or Fisher exact tests. Concordance between tests was measured using the kappa coefficient.

Cost analysis

Unit costs for both microscopic methods (LED and ZN) were estimated based on a routine diagnostic algorithm implemented at the study site with ~20 specimens processed per batch. All economic costs associated with each respective system was analyzed in health services perspective, where we concentrated on laboratory-only costs [12, 13]. Unit costs were calculated using the 'ingredients' approach, and multiplying the quantity of inputs used by price [14]. All capital costs (laboratory space and equipment) were annualised based on their estimated expected life-years. Overhead costs were calculated by fractionating staff costs and time, and space and infrastructure utilized to each test [14] All pricing and costs are expressed in 2009-2010 U.S. Dollars based on the currency exchange rates at the time writing. Overhead costs used in this analysis were provided by the National Health Laboratory Services (NHLS).

Results

A total of 647 sputum samples were collected from 345 patients. 295 patients consented to HIV testing. 88 patients (29.8%) were HIV-infected with a median (IQR) CD4 count of 178 (124-320) cells/ml. 50 patients either refused HIV testing or had unavailable results and were exclude from analysis. The mean (SD) age of patients was 36 (7) years, the majority were male and black African and 34.5% had a history of previous TB. Of the 647 samples cultured, 25 were contaminated and non-tuberculous mycobacteria were isolated from 5, leaving 617 evaluable cultures. Of these, 161 (26%) were positive for *M.tb*.

Table 1 shows the performance characteristics of LED microscopy compared with ZN light microscopy using unprocessed sputum and conventional MVFM using concentrated samples, and stratified by HIV-infection and CD4 count. The overall sensitivity of LED and ZN microscopy on direct smears was similar (~50%) with an agreement of 97% (κ=0.871), while on concentrated samples LED microscopy and MVFM were almost identical (66%) with an agreement of 97% (κ=0.896). The sensitivity of LED and MVFM was better in concentrated vs. unconcentrated samples (66% vs. 52% respectively, p =0.005).

In HIV infected patients, the sensitivity of all 4 microscopy modalities decreased compared to HIV un-infected patients, and the performance of MVFM on concentrated samples was significantly better in HIV un-infected compared to HIV-infected patients (46%(32-61) vs. 74%(64-82),p=0.002). However, in both unconcentrated and concentrated sputum samples the performance of LED FM, although decreased in the HIV-infected sub-group, did not differ significantly between HIV-infected and HIV-uninfected groups [(unconcentrated samples, 57% vs. 46%, p=0.28),(concentrated samples, 71% vs. 54%, p=0.06)].. Amongst HIV-infected patients, the sensitivity of LED microscopy was better than MVFM on concentrated samples although not reaching significance (54% vs. 46%, respectively, p=0.5; Table 1). In HIV infected patients with CD4 counts <200 cells / ml, the sensitivity of LED microscopy was better than MVFM microscopy but did not reach significance (56% vs. 44% respectively, p=0.5).

The median (IQR) time for reading unconcentrated smears was significantly quicker with LED FM microscopy compared to standard ZN light microscopy [1.8 (1.7-1.9)minutes vs.2.5 (2.2.-2.7), p=<0.001]. The mean time saved by using LED compared to ZN was 25%. Reading concentrated smears took 35% less time than unconcentrated smears using either LED or MVFM.

Average unit costs, expressed as cost per slide read, was cheaper for LED based methods (U$1.63) as compared to conventional light microscopic method using ZN staining (U$2.10; see Table 1B). Most of the cost-savings were as result of reduced time required for reading slides and simpler staining process. To screen 1000 TB suspects on their first

sputum sample and using concentration methods, LED and ZN would cost $1568 and $2049, respectively.

Discussion

This is, to our knowledge, the first study that comprehensively examines the usefulness and accuracy of LED microscopy in HIV-infected persons. The major finding of this study is that LED-microscopy, despite being cheaper, performs as well as ZN-microscopy or MVFM in HIV-infected persons using both concentrated and unconcentrated sputum samples. In fact, LED microscopy performed better than ZN staining when using uncentrifuged samples, and better than MVFM when using centrifuged samples though the difference failed to reach significance. The density of mycobacteria is lower in the sputa of TB-HIV co-infected patients who have pauci-bacillary disease and thus it is important to confirm that LED microscopy performs as well as other methodologies in this sub-group of patients.

Published studies have already shown that LED microscopy performs as well as conventional microscopy and MVFM in unselected patients with TB in both research and operational settings [6-8, 15]. Marais et al showed a slightly better, although not statistically significant, detection rate using LED as compared to MVL (5% vs 12%, n= 221), while van Hung et al reported slightly lower sensitivity of LED microscopy, which they likely attributed to photo-bleaching, as the smears were read on a MVL microscope before the LED microscope. However, there are hardly any data in TB-HIV co-infected persons and the published WHO guideline does not address performance in this subgroup of patients, although WHO recommendations are meant apply to both HIV infected and un-infected TB suspects. Confirming efficacy in HIV-TB co-infected patients is important to the roll-out of LED microscopy by National TB Programmes (NTPs) in African countries where this technology is most needed and where up to 80% of patients have TB-HIV co-infection, and the electricity supply is erratic and dark-room facilities limited. Thus, these data may enhance and facilitate the widespread uptake of LED microscopy in Africa.

The second major finding is that in TB-HIV co-infected patients with a CD4 counts less than 200 cells/ml LED-microscopy performs as well as ZN-microscopy or MVFM using both unconcentrated and concentrated sputum samples. A similar pattern of the superiority of LED microscopy over other microscopy modalities was seen in this subgroup. This finding is significant given that the majority of TB-HIV co-infected patients presenting to services in Africa have a CD4 count of less than 200 cells/ml [16].

Thirdly, there are no published cost analysis data of LED microscopy, although studies have shown that FM is a cost-effective alternative to ZN in resource-limited settings [17] We show that LED-microscopy using auramine staining is cheaper than conventional microscopy using ZN staining. This information will be crucial to enhance uptake of this newer technology by policymakers, especially since WHO recently endorsed LED microscopy for widespread use.

A limitation of our study is the lack of sufficient numbers of HIV infected patients to be able to demonstrate superiority of LED microscopy over conventional microscopy or MVFM. However, at the very least, there is no evidence of reduced sensitivity or specificity when using LED microscopy to read smears from HIV-infected individuals. We did not perform MVFM on unconcentrated samples because of workload considerations and because MVFM is usually done only on concentrated specimens in most settings. To avoid the effect of photo-bleaching which biases against LED microscopy we elected to use separately prepared slides for each form of microscopy.

In 2010, the WHO recommended that conventional fluorescence microscopy be replaced by LED microscopy using auramine staining [5]. However, there data are hardly any data about applicability in HIV-infected persons. Our data inform on this gap in knowledge. Our findings, given the superior performance of LED microscopy and its user, field and cost-friendly format, suggest that African NTPs should now initiate and accelerate the roll-out of LED microscopy. This will enhance the availability of fluorescent microscopy in resource-poor settings and thus impact on case detection rates and lowering of disease

burden. Studies are also required to evaluate the combination of other microscopy-enhancing methodologies (e.g. TB-Beads [18] which is a field-friendly concentration technique that obviates the use of a centrifuge) with LED microscopy so that ease of use in resource-poor settings is further improved.

Acknowledgements:
This work was supported by grants from European Commission (TBSusgent; EU-FP7), EDCTP, and Canadian Institutes of Health Research (MOP-89918).

JP is supported by a Discovery Foundation Fellowship, the Fogarty International Clinical Research Scholars/Fellows Support Centre National Institutes of Health grant R24TW007988 and SATBAT. KD is supported by the South African NRF Research Chairs Initiative (SARChI), and an MRC Career Development Award. MP is supported by a CIHR New Investigator Award.The authors have no conflicts of interest.

References

1. WHO/TDR. **Diagnostics for tuberculosis: global demand and market potential.** 2006: [http://www.who.int/tdrold/publications/publications/tbdi.htm – accessed 5 November 2011].
2. Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, *et al.* **Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review**. *Lancet Infect Dis* 2006,6:570-581.
3. Anthony RM, Kolk AH, Kuijper S, Klatser PR. **Light emitting diodes for auramine O fluorescence microscopic screening of Mycobacterium tuberculosis**. *Int J Tuberc Lung Dis* 2006,10:1060-1062.
4. Minion J, Sohn H, Pai M. **Light-emitting diode technologies for TB diagnosis: what is on the market?** *Expert Rev Med Devices* 2009,6:341-345.
5. WHO. **Fluorescent light emitting diode (LED) microscopy for diagnosis of tuberculosis - policy statement**. 2010:http://www.who.int/tb/dots/laboratory/who_policy_led_microscopy_july10.pdf.
6. Marais BJ, Brittle W, Painczyk K, Hesseling AC, Beyers N, Wasserman E, *et al.* **Use of light-emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum**. *Clin Infect Dis* 2008,47:203-207.
7. Van Deun A, Chonde TM, Gumusboga M, Rienthong S. **Performance and acceptability of the FluoLED Easy module for tuberculosis fluorescence microscopy**. *Int J Tuberc Lung Dis* 2008,12:1009-1014.
8. Trusov A, Bumgarner R, Valijev R, Chestnova R, Talevski S, Vragoterova C, Neeley ES. **Comparison of Lumin LED fluorescent attachment, fluorescent microscopy and Ziehl-Neelsen for AFB diagnosis**. *Int J Tuberc Lung Dis* 2009,13:836-841.
9. Kent PT, Kubica GP. **Public health mycobacteriology: Guide for the Level III Laboratory.** In. Edited by Services UDoHaH: Centres for Disease Control, USA,; 1985.
10. WHO. **Laboratory Services in Tuberculosis Control. Part 2: Microscopy**. In; 1998: [http://whqlibdoc.who.int/hq/1998/WHO_TB_1998.1258_(part1992).pdf – accessed 1995 November 2011]
11. De Wit D, Steyn L, Shoemaker S, Sogin M. **Direct detection of Mycobacterium tuberculosis in clinical specimens by DNA amplification.** *J. Clin Micro* 1990,28:2437-2441.
12. Drummond MF, O B, B., Stoddart GL, Torrance GW. **Methods for the Economic Evaluation of Health Care Programmes**. Second Edition ed. Oxford: Oxford University Press 1997.
13. Tan-Torres Edejer T, Baltussen R, Adam T, Hutubessy R, Acharya A, Evans DB, Murray CJL. **Making Choices in Health: WHO Guide to Cost-effectiveness Analysis**. In; 2003.
14. Sohn H, Minion J, Albert H, Dheda K, Pai M. **TB diagnostic tests: how do we figure out their costs?** *Expert Rev Anti Infect Ther* 2009,7:723-733.

15. Shenai S, Minion J, Vadwai V, Tipnis T, Shetty S, Salvi A*, et al*. **Evaluation of light emitting diode-based fluorescence microscopy for the detection of mycobacteria in a tuberculosis-endemic region**. *Int J Tuberc Lung Dis* 2010,15:1-6.
16. Holmes CB, Wood R, Badri M, Zilber S, Wang B, Maartens G*, et al.* **CD4 decline and incidence of opportunistic infections in Cape Town, South Africa: implications for prophylaxis and treatment**. *J Acquir Immune Defic Syndr* 2006,42:464-469.
17. Sohn H, Sinthuwattanawibool C, Rienthong S, Varma JK. **Fluorescence microscopy is less expensive than Ziehl-Neelsen microscopy in Thailand**. *Int J Tuberc Lung Dis* 2009,13:266-268.
18. Wilson S, Lane A, Rosedale R, Stanley C. **Concentration of Mycobacterium tuberculosis from sputum using ligand-coated magnetic beads**. *Int J Tuberc Lung Dis* 2010,14:1164-1168.

Study group (n=number of sputum samples)	Unconcentrated sputum sample		Concentrated sputum pellet		Unconcentrated sputum sample		Concentrated sputum pellet	
	Sensitivity		Sensitivity		Specificity		Specificity	
	ZN (95% CI)	LED (95% CI)	MVFM (95% CI)	LED (95% CI)	ZN (95% CI)	LED (95% CI)	MVFM (95% CI)	LED (95% CI)
All (n=616)	49[*#] (42, 57)	52[*@] (44, 59)	66 (58, 73)	66[#@] (58, 73)	99 (98, 100)	100 (98, 100)	99 (98, 100)	99 (98, 100)
HIV Negative (n=377)	55[a] (45, 65)	57[c] (46, 66)	74[e] (64, 82)	71[d] (61, 79)	99 (97, 100)	99 (97, 100)	99 (97, 100)	99 (97, 100)
HIV Positive (n=150)	39[$,b,a] (26, 54)	46[$,c] (32, 61)	46[&,e] (32, 61)	54[b,&,d] (39, 68)	100 (97, 100)	100 (97, 100)	100 (97, 100)	99 (95, 100)
CD4 < 200cells/ml (n=69)	39 (20, 61)	50 (29, 71)	44[^] (25, 66)	56[^] (34, 75)	100 (93, 100)	100 (93, 100)	100 (93, 100)	100 (93, 100)

ZN= Ziehl-Neelsen staining light microscopy, LED = Auramine-O staining light emitting diode fluorescent microscopy, MVFM = Auramine-O staining conventional mercury vapour fluorescent microscopy
P values: *p=0.33, [#]p=0.001, [@]p=0.005, [$]p=0.25, [a]p=0.08, [b]p=0.092, [&]p=0.5, [^]p=0.5, [c]p=0.28, [d]p=0.06, [e]p=0.002,

Table 1. Sensitivities (%), specificities (%), PPV (%) and NPV (%) with 95% confidence intervals for each of the four different smear detection methods stratified by HIV status and CD4 cell count. Culture positivity to *M. Tuberculosis* served as the reference standard.

.

Type of Cost / Type of Microscopy	**Overhead**	**Building Space**	**Equipment**	**Staff**	**Reagents and Chemicals**	**Consumables**	**Total**
LED (Lumin)	0.81	0.01	0.08	0.47	0.04	0.22	1.63
ZN	1.06	0.01	0.08	0.69	0.04	0.22	2.10

Table 2. Average unit cost per AFB smear on sputum sample/slide: LED with auramine vs. Ziehl-Neelsen (ZN) light microscopy

[Overhead: maintenance, running, management and supervision costs | Building: cost relating to the use of specific physical (laboratory space) for procedures relevant for microscopy | Equipment: costs based on annualized cost of laboratory equipment, inclusive of procurement costs | Staff: complete staff hands-on time from the receipt of specimen to dispatch and filing of the result forms | Chemicals and Reagents: based on costs of ready-made staining reagents procured by NLHS and approximately 3 ml of use per slide for each staining reagent | Consumables: cost associated with the use of general consumables such as sputum collection cups, gloves, glass slides, etc.]